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Shorter Notes

Agravitropic Growth of the Early Leaves of Apogamous Sporophytes of *Dryopteris tyrrhena*.—*Dryopteris tyrrhena* Fraser-Jenk. & Reichst., a Western Mediterranean endemic, is a threatened species represented by a few populations in Spain, France, and Italy (Magrini and Scoppola, Inform. Bot. Ital. 42(2):595–597. 2010). The relict type of its distribution (rare and scattered with big gaps between different localities, mostly in crevices and caves) suggests that *D. tyrrhena* is an old species of the Tertiary flora of the Mediterranean mountains (Fraser-Jenkins *et al.*, Fern Gaz. 11(2–3):177–198. 1975; Bernardello and Martini, *Felci e piante affini in Liguria e in Italia*. Le Mani-Microart's Edizioni, Recco-Genova. 2004). It is an allotetraploid species (2n=164) originated by interspecific hybridization between the diploid species *D. oreades* Fomin (2n=82) and *D. pallida* (Bory) Maire & Petitm. (2n=82) with subsequent chromosomes doubling (Fraser-Jenkins *et al.*, 1975).

This study on the in vitro development of apogamous sporophytes of *Dryopteris tyrrhena* (Magrini, *Plant Biosystems* 145(3):635–637. 2011; Magrini *et al.*, *Studi Trent. Sci. Nat.* 90:165–169. 2012) was undertaken in summer 2008 at the Tuscia Germplasm Bank of the Botanic Gardens of Viterbo (Italy) in order to learn about its reproductive biology, and for conservation purposes, to obtain information on the biological factors that may have contributed to the strong fragmentation of its distribution. Fresh spores were collected in September 2007 from a wild population of *D. tyrrhena* growing within the Cinque Terre National Park (Riomaggiore, La Spezia, Italy). Studies of gametophyte and sporophyte development were carried out according to the protocol of Menendez *et al.* (*Plant Cell Rep.* 25:85–91. 2006; Quintanilla and Escudero, *Ann. Bot.* 98:609–618. 2006; Magrini *et al.*, 2012). All the spores were separated from sporangia using sieves with a mesh size of 71 μm, and then were soaked in Eppendorf tubes with 1.5 ml of distilled water for 24 h. After, they were surface sterilized for 3 min. in a 0.5% NaOCl solution, supplemented with a drop of Tween 20 to improve the efficiency of the sterilization. They were then rinsed three times with sterile distilled water and centrifuged at 6,000 rpm for 3 min. between rinses. The spores were sown with three replicates in sterile plastic Petri dishes (6 cm diameter) containing 15 ml of MS medium (Murashige and Skoog, *Physiol. Plant.* 15:473–497. 1962) (*PhytoTechnology Laboratories*® Shawnee Mission, KS, USA), supplemented with 0.7% agar (Plant tissue culture grade, *AppliChem*, Darmstadt, Germany) and a Nystatin solution (100 Uml^{-1}), which was added as a fungicide (*AppliChem*, Darmstadt, Germany). The cultures were maintained under cool-white fluorescent illumination (Osram Dulux L 36W/840 Lumilux, 2900 lm), a 12-h photoperiod, and a temperature of 20±1°C (Sheffield *et al.*, Amer. Fern J. 91(4):179–186. 2001; Magrini *et al.*, 2012). The dishes were examined daily for spore germination (defined as the first emergence of the rhizoid) and weekly for gametophyte growth and sporophyte development.

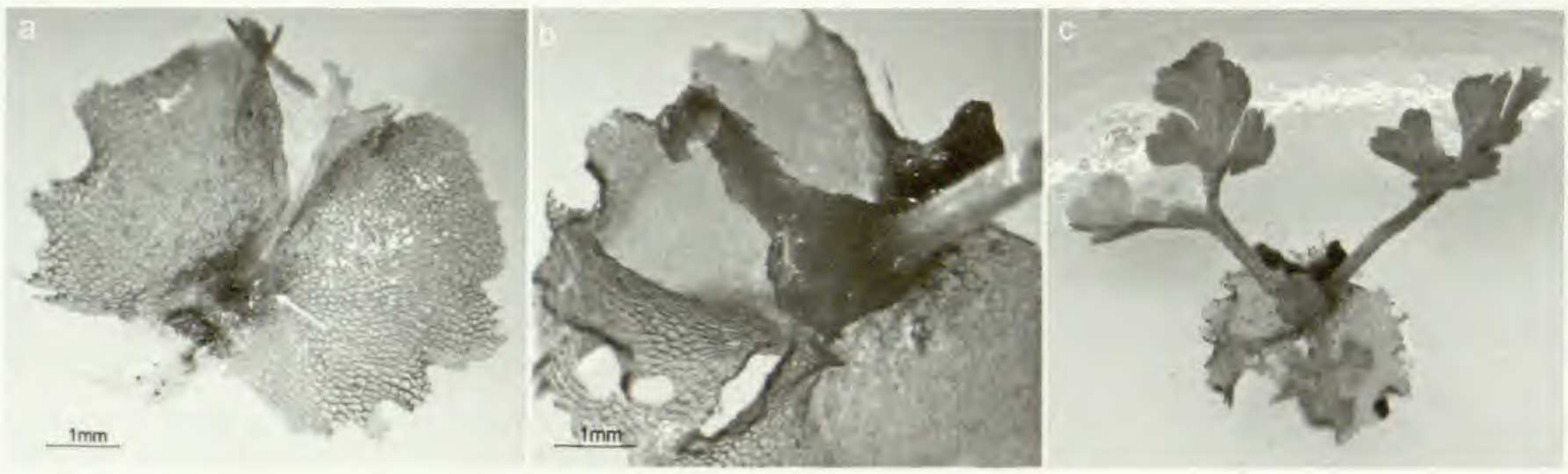

FIG. 1. a) Cordate gametophytes of *Dryopteris tyrrhena*, with rhizoids in the ventral side and an apogamous bud in the dorsal side, in the cushion area behind the apical notch. b) First fronds arose from the apogamous bud, showing circinate vernation. c) Apogamous sporophyte with young pinnate fronds grown upward, each diverging at an angle of about 100° from the previous one.

A low percentage (<5%) of the spores germinated after 36 days after sowing. All gametophytes were filamentous at the beginning and they followed a normal trend of development in the transition from protonema to the laminar-cordate phase: the first oblique division of the terminal cell of the germ filament started after the fourth longitudinal division (Raghavan, *Developmental biology of fern gametophytes*. Cambridge University Press. 1989), resulting in laminar heart-shaped gametophytes 15 days after germination. All the cordate gametophytes produced rhizoids in the ventral side and developed apogamous buds in the dorsal side, in the cushion area behind the apical notch (Fig. 1a), about 60–70 days after germination (Magrini *et al.*, 2012). This same pattern has been previously recorded for *D. affinis* (Lowe) Fraser-Jenk. subsp. *affinis* (Menéndez *et al.*, 2006). Sporophytes arose from these buds in a few days, with young pinnate fronds, which started to quickly grow, showing circinate vernation (Fig. 1b).

During the apogamous sporophyte development, an interesting phenomenon was noticed. In the beginning, the leaves grew upward, each diverging at an angle of about 100° from the previous one (Fig. 1c), as has been observed by

FIG. 2. Agravitropic response: some leaves developed downward and they grew into the agar of the culture medium.

Duncan (Bot. Gaz., 105:202–211, 1943) for certain apogamous forms of *D. affinis* (Lowe) Fraser-Jenk. subsp. *borreri* (Newman) Fraser-Jenk. An anomalous growth was observed a few days later as the leaves showed an agravitropic response. They developed both upward and downward, growing also into the agar of the culture medium (Fig. 2). Other studies are testing the already known induction factors of apogamy, like the presence of sucrose in the culture medium, high light intensity, and the vertical growth of the gametophytes (Magrini *et al.*, 2012). Their preliminary results led us to hypothesize that apogamy may have been induced by ethylene production during *in vitro* culture (Elmore and Whittier, Can. J. Bot. 53:375–381, 1975). In fact, the dishes were sealed with Parafilm, in order to reduce contamination, to prevent excessive water loss, and to reduce air exchange. Menéndez *et al.* (2006) showed how auxins play a stimulatory role during the induction and differentiation of apogamous embryo development in *D. affinis*. Van der Laan (1934) was the first to consider the possibility that ethylene acts on auxin in plants. Given its potential role in apogamy, it is possible that ethylene could also be influencing the normal gravitropic response. However, more experimental work is needed to study the influence of ethylene on tropisms and to clarify the effective cause of this agravitropic response.—Sara Magrini, Tuscia Germplasm Bank, Botanic Gardens of Viterbo, Tuscia University, largo dell'Università, Blocco C, 01100, Viterbo, Italy, e-mail: magrini@unitus.it, and Anna Scoppola, Department of Agriculture, Forestry, Nature, and Energy, Tuscia University, via S. Camillo de Lellis, 01100, Viterbo, Italy, e-mail: scoppola@unitus.it.